ANNUAL REPORT 1948-49

Project No. 742. The nature and action of the gene in bacteria. Lederberg (Mrs. Esther Lederberg, National Cancer Institute, Junior Fellow, and University Fellow; Norton Zinder, Ethelyn Lively, Research Assistants Donald A. Gordon, Project Assoc.) State funds, W.A.R.F., Rockefeller Foundation, National Institute of Health.

The research undertaken in this field can be reviewed under the following headings:

1. Genetic aspects of life cycle in Escherichia coli; cytological studies. (Miss Lively)

2. Genetic control of fermentation enzymes in E. coli.

3. Genetic control of mutability. Concluded. (Mrs. Lederberg)

4. Lysogenic bacterial virus in E. coli. (Mrs. Lederberg)

5. Genetic recombination in Salmonella and E. coli. (Mr. Zinder)

1. Genetic aspects of life cycle in Escherichia coli: cytological studies. (Miss Lively)

The study of the genetic behavior of heterozygous diploid stocks of E. coli, obtained in crosses of strain K-12 of this organism, has been continued, without marked clarification, as yet, of the aberrant segregation behavior of these diploids, namely that various segregant types are thrown off at frequencies markedly different from the theoretical 1:1 for each character pair. Direct evidence that this aberration is due, in part, to a chromosome deficiency heterozygous in the diploid, has been obtained by examination of diploids which were pure for maltose fermentation, although one parent was Mal+, the other Mal-. If the failure of such diploids to segregate for maltose fermentation were due to the presence of a deficiency including the Mal+ gene, then reverse mutations for maltose fermentation in such stocks would lead to pure (not segregating) Mal+ from pure Mal-. This was found to be the case. On the other hand, comparable experiments with pure lactose-negative diploids, obtained when both parents were Lac-, resulted in reverse mutations which segregated for lactose fermentation. The experiment can be illustrated: Mal-/deficiency $\xrightarrow{R.M.}$ Mal+/ deficiency (pure Mal+), but Lac-/Lac- (diploid) $\xrightarrow{R.M.}$ Lac-/Lac+ (i.e., segregating for Lac). Additional evidence bearing on the structure of the aberrant diploids has come out of single cell pedigree studies with the collaboration of Dr. M. R. Zelle of Cornell University. In these pedigrees, inviable cells occur rather frequently, and apparently correlated with reduction from diploid to haploid. We may conclude, therefore, that the aberrant segregation ratios are a result of the inviability of approximately half the segregants. Were it not for crossing-over, all of the viable segregants would be of the same type, but to the extent that a gene can cross-over with the deficient region, a haploid segregants bearing the alternative allels can be recovered. This conclusion is supported by a gradual variation in segregation ratio which parallels the linkage distance of a given factor from the deficient region on the chromosome map.

These considerations are probably important for crosses in which haploid recombinants are isolated as well. This is best illustrated by the fact that the genetic factors controlling maltose-fermentation and streptomycin-resistance which show aberrant behavior (i.e., deficiency) in the diploids, also prove to be unmappable in haploids. We may infer, therefore, that the meiotic mechanism is generally unstable, and that segmental losses are occurring, particularly for the region containing the Mal and streptomycin-resistance genes, in all zygotes, whether they undergo immediate segregation and give only haploid progeny, or are delayed in their segregation so that heterozygous diploids are recovered. The detailed chromosomal mechanism which underlies this meiotic instability is not yet understood, but is the object of current research.

In hopes of throwing further light on the genetic cycle in E. coli, a cytological study has been initiated, particularly with the object of comparing the haploid and diploid cultures. Definite differences have been found, so that the genetic status of a culture can be predicted from its cytological appearance, but we have not yet been able to interpret the slides. Attached is an enlarged photomicrograph illustrating the nuclei of typical haploid cultures; comparable prints of diploid cultures are not presently available.

2. Genetic control of fermentation enzymes in E. coli.

The study of E. coli lactase, or B-galactosidase, as a model system for gene enzyme relationships, has been continued. In the course of kinetic and adaptation studies on intact cells, considerable variation was found from day to day, which was ultimately traced to the fact that washed cells of K-12, although stable in distilled water, are autolysed upon standing in M/15 or higher phosphate buffer and other salts. The autolysis can be detected as an appreciable reduction in the optical density of a cell suspension. Concomitant with the autolysis is a striking increase in the apparent enzymatic activity of the suspension, which may reach as high as thirty-fold. In addition, a small fraction, about 20%, of of the activity is released from the cells to the buffer. This result indicated

that extreme caution would be needed in studies on intact cells, and further work on adaptation rates and genetic effects would have to wait upon the development and verification of methods of assaying cells so that the values obtained would have some meaning.

Upon further examination, it was found that a variety of substances were even more effective than buffer in induced this "autolytic activation" of the enzyme. Thymol, benzene, and caprylic alcohol and desiccation over P_2O_5 were especially effective, and fairly reproducible and optimum amounts of activation could be obtained with these agents, although entirely satisfactory conditions have not yet been worked out. In addition, soaps, gramicidin, and other detergents have considerable activity, but they erratically inactivate the released enzyme. The simplest interpretation of the activation is that the cell contains a diffusion barrier, which impedes the transport of the substrate to the enzyme. Whether this barrier is simply the cell wall, or a more subtle system comparable to the "cytophorase" units of other cells, is not yet clear. As mentioned in the previous report, the enzyme in intact cells has different kinetic properties from that in soluble extracts, which may be a reflection of the kinetics of the transport mechanism rather than of the enzyme in situ.

With the help of the amplification of activity induced by these autolytic agents, a clear demonstration that lactase was not entirely adaptive became possible. That is, cells grown on glucose give extracts whose lactase activity was as much as 1% of fully adapted cells grown on lactose. A reinvestigation of this property showed that very considerable amounts of lactose (ca 10-15% of full adaptation) were present in cells grown on other sugars, e.g., maltose, or on non-carbohydrate carbon sources, e.g., sodium succinate or peptone. Thus it would appear that glucose has the special property of largely suppressing the elaboration of lactase, while on other carbon sources, fairly large amounts of nonadaptive lactase are formed. The distinction between adaptive and constitutive enzymes is further blurred by these experiments.

As far as could be told, the extent of autolytic activation is the same in cells containing varying amounts of lactase. That is, the maximum ratio of activity in autolysed compared to intact cells was uniformly about 30. This observation clears up the very puzzling and widely cited observations of Deere who, in 1939, reported that enzymatic adaptation to lactose was entirely spurious inasmuch as unadapted cells could be "adapted" merely by drying or desiccating them. He was, however, owing to technical limitations, unable to test these effects on cells grown on lactose. The activation of adapted cells to the same extent, 30x, in the current experiments, leaves little doubt that cells grown on lactose do adapt by synthesising more enzyme. Equally, the differences lactose-positives and lactose-negative mutants, some of which synthesize small amounts of lactase, do not depend on permeability differences, but on differences in their capacity to synthesize lactase.

3. Genetic control of mutability. Concluded. (Mrs. Lederberg)

During 1947-48 a number of lactose-negative stocks of E. coli were isolated which were unusually stable as far as their revertibility to Lac+ was concerned. Some of these stocks were of particular interest because, in crosses with wild type, they gave some mutable lactose-negative progeny. This result implies that some genetic factors, which can be separated from the Lac- locus by recombination, may be able to influence the true or apparent mutability of the Lac- genes. In a number of cases which have now been analysed, it appears likely that the modifying genes are not influencing the true mutability of the Lac loci, but are influencing the manifestation of the mutations which do occur, so that few or none of them are observed. The most characteristic modification is that exerted by a number of mutations which interfere with galactose-fermentation (Gal-). The effect of the Gal- modifier is two-fold. Firstly, Gal- interferes to some extent with the fermentation of lactose, so that on a lactose medium, Lac+ mutations from Lac- do not have as decisive a selective advantage. Secondly, many of the Gal- mutations have an inverse effect on the Lac- stock, making the latter better

able to ferment lactose. The biochemical basis of these effects is not well understood, but their overall result is to make the difference in selective advantage between Gal-Lac- and Gal-Lac+ mutants insufficient to ensure the uniform manifestation of such reverse mutants on the lactose selective medium.

4. Lysogenic bacterial virus in E. coli. (Mrs. Lederberg)

The sexually fertile strain, K-12, of E. coli that has been used in many of the reported experiments has been found to carry a latent bacterial virus, opening up an investigation of the genetic effects and properties of latent virus. As mentioned in last year's report, lysogenicity has been a serious problem in Salmonella genetic work, so that it has been especially important to learn what we could about it.

As in plants (e.g., potatoes), latent bacterial viruses can only be detected with the help of sensitive "indicator" cultures, for they usually have no obvious effect on their carrier host. In K-12, such an indicator culture appeared as a "mutant" in a population treated with ultraviolet light. Attempts to disinfect K-12 with ultraviolet under experimentally controlled conditions have however been disappointing.

The likeness between bacterial lysogenicity and latent plant viruses is emphasized by the behavior of the virus in crosses. In plants, latent virus is ordinarily not transmitted to seed. In E. coli K-12, a considerable proportion of sexual progeny are uninfected with and sensitive to the latent virus, provided one parent is uninfected. However, when both parents are infected, all or almost all of the sexual progeny are infected also. The results speak for some sort of interruption of virus multiplication or transmission at the sexual stage, but the details have yet to be uncovered.

5. Genetic recombination in Salmonella and E. coli. (Mr. Zinder)

In hopes of learning the scope and extent of gene recombination among bacteria, a systematic survey is being made of coliform bacteria and of the related Salmonella group to determine whether recombination occurs in these forms.

A distinct strain of E. coli, isolated from chicken feces by Drs. Shapiro and Sarles, has been found which crosses with E. coli K-12, but the yields of recombinants have been extremely low, possibly because the chicken strain secretes an antibiotic active against K-12. However, resistant mutants K-12 have just been isolated, and these should permit of more effective crossing of the two strains. It is of interest that one of these strains is sucrose+, the other sucrose-, showing that the varietal differentiation of E. coli into communis and communior is of very little genetic significance, and likely rests merely on a single gene mutation. A number of Salmonella strains have given results strongly suggestive of recombination, but this conclusion has not yet been adequately confirmed.

For such a survey it might be desirable to have a technique of isolating recombinants accessory to that which depends on nutritional requirements. Drug resistance was found to be a useful basis for recombination studies. Two mutants of E. coli K-12, one resistant to sodium azide, the other two streptomycin, were obtained. Recombinants between the mutants were selected by plating mixed cultures into agar containing both azide and streptomycin. That the doubly resistant organisms were recombinants was verified by checks on other markers (fermentative, nutritional and phage-resistance), which frequently had recombined also, although they were not constrained to do so by the selective medium itself.

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December 1949